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(54) NOVEL FUSED PROTEIN, GENE THEREFOR, RECOMBINANT VECTOR, RECOMBINANT VIRUS, AND ITS USE

(57) A DNA coding for a fusion protein comprising a polypeptide having the antigenicity of Mycoplasma gallisepticum and a polypeptide derived from Herpesvirus outer membrane protein, in which the polypeptide derived from the outer membrane protein has been ligated with the polypeptide having the antigenicity of Mycoplasma gallisepticum at the N terminus thereof, is prepared. The DNA is inserted into a region non-essential to growth of Avipox virus and the resulting recombinant Avipox virus is provided as a more potent recombinant virus as an anti-Mycoplasma vaccine.

Description

TECHNICAL FIELD

[0001] The present invention relates to a novel fusion polypeptide of a polypeptide having the antigenicity of Mycoplasma gallisepticum and a polypeptide derived from the outer membrane protein of herpes viruses, a hybrid DNA coding for the fusion polypeptide, and a recombinant Avipox virus bearing the hybrid DNA, as well as a vaccine using the recombinant Avipox virus.

BACKGROUND ART

[0002] Mycoplasma gallisepticum (hereinafter sometimes abbreviated as MG) is a bacterium that causes reduction in an egg-laying rate and a hatching rate of eggs for poultry including chicken. This causative MG is widely spread all over the world so that a great deal of damage has been done to the poultry farming. For the prevention of MG, an inactivated vaccine or a live vaccine is currently utilized. However, the former live vaccine involves disadvantages of complicated inoculation procedures, short duration of immunity, expensive etc. The latter vaccine has such a defect that an unexpected disease might be developed by use in combination with live vaccine for other disease. Another disadvantage is that MG agglutination reaction system, which makes rapid detection of MG infection possible, can not be used for both inactivated and live vaccines.

[0003] It is expected that a protein derived from MG such as its antigenic protein for preventing from MG infection would be produced by genetic engineering technology and utilized as a vaccine.

[0004] The production system of the antigenic protein of Mycoplasma gallisepticum using E. coli or yeast by means of genetic engineering (JPA 2-111795, etc.) encounters such problems that depending upon a protein to be expressed, the antigenic protein is only expressed in a less amount, proteins of host origin might be by-produced and intermingled, host-derived pyrogen is removed only with difficulty, etc. For these reasons, studies are still focused on a recombinant virus to prepare antigenic proteins or on a recombinant live vaccine.

[0005] The expression of foreign genes using recombinant viruses, in most cases, genes of eucaryotes or viral genes are expressed. For this reason, addition or expression mode of sugar chains or the like is similar to the protein expression mechanism in infected cells. Thus, induction of an antibody titer to the expressed protein was relatively easy in vivo. However, genes of prokaryotes are rarely expressed in recombinant viruses. Because of different expression mode between eukaryotes and prokaryotes, it was difficult to say that a specific antibody was effectively induced (Austen et al., Protein Targeting and Selection, Oxford Univ. Press (1991)).

[0006] Turning to MG, recombinant viruses in which a gene coding for the protein has been incorporated are known by JPA 5-824646 and JPA 7-133295, WO 94/23019, etc. In particular, WO 94/23019 reveals that when a recombinant virus capable of expressing the antigenic protein of MG having a viral membrane anchoring region, which is obtained by ligating the signal membrane anchoring portion of HN gene of New Castle disease virus (hereinafter abbreviated as NDV) with the antigenic gene of MG, is inoculated as a recombinant live vaccine, the antibody is induced more effectively than a recombinant virus capable of expressing the antigenic gene of MG alone.

[0007] However, expression to such an extent is not always sufficient to achieve the desired effect as a vaccine.

[0008] Therefore, it is the urgent need to find an improved method for higher recognition of the antigen in order to develop an effective vaccine against MG infections.

[0009] Outer membrane proteins other than NDV mentioned above are known also in the genus Herpesvirus, etc. With respect to glycoproteins B(gB), C(gC), D(gD), H(gH) and I(gI) of herpes simplex viruses; proteins gBh, gCh, gDh, gHh and gIh of Marek's disease viruses (hereinafter often referred to as MDV) corresponding to herpes simplex virus glycoproteins gB, gC, gD, gH and gI and proteins of the genus Herpesvirus homologous to those proteins described above, etc., the nucleotide sequence and amino acid sequence of these proteins are known. It is also known that a part of these proteins induces neutralizing antibodies of herpes simplex viruses (Deluca et al., Virology, 122, 411-423 (1982)). It is further known that neutralizing antibodies can be induced by incorporating genes coding for these proteins into vaccinia viruses and expressing the genes (Blacklaws et al., Virology, 177, 727-736 (1990)).

[0010] However, investigations to make use of signal sequences of such outer membrane proteins of the genus Herpesvirus were hardly made so far.

DISCLOSURE OF THE INVENTION

[0011] Under the situation of the prior art stated above, the present inventors have made extensive studies to provide a recombinant virus capable of expressing a Mycoplasma antigenic protein having an enhanced infection prevention activity in large quantities, which allows a host to recognize the antigen highly efficiently. As a result, it has been found that by infecting to a host a recombinant Avipox virus, in which a hybrid DNA obtained by ligating a DNA of the outer

membrane protein of the genus Herpesvirus with a DNA of the antigenic protein of Mycoplasma has been inserted, the antigen recognizing ability of the host can be markedly improved. The present invention has thus been accomplished.

[0012] Accordingly, the present invention provides:

a fusion protein comprising a polypeptide having the antigenicity of Mycoplasma gallisepticum (hereinafter sometimes referred to as Mycoplasma-derived polypeptide) and a polypeptide derived from the outer membrane protein of a herpes virus (hereinafter sometimes referred to as Herpesvirus-derived polypeptide) characterized in that the polypeptide derived from outer membrane protein is ligated with the polypeptide having the antigenicity of Mycoplasma gallisepticum at the N terminus thereof;
a hybrid DNA coding for the fusion protein;
a recombinant Avipox virus in which the hybrid DNA has been incorporated; and,
a live vaccine comprising the recombinant Avipox virus as an effective ingredient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013]

Fig. 1 is a drawing for explaining procedures for construction of pNZ40K-S.
Fig. 2 is a drawing for explaining procedures for construction of pNZ40K-S.
Fig. 3 is a drawing for explaining procedures for construction of pNZ40K-S.
Fig. 4 is a drawing for explaining procedures for construction of pNZ40K-C.
Fig. 5 is a drawing for explaining procedures for construction of pNZ40K-C.
Fig. 6 is a drawing for explaining procedures for construction of pNZ40K-C.
Fig. 7 shows the results of Western blotting by which expression of TTM-1 polypeptide was confirmed.
Fig. 8 shows scores of the tracheal legion caused.

BEST MODE FOR PRACTICING THE INVENTION

Mycoplasma-derived polypeptides and genes therefor

[0014] In the present invention, the term Mycoplasma-derived polypeptides is used to mean the antigenic proteins that cause an antigen-antibody reaction with MG immune serum or MG infected serum and that are derived from MG. These polypeptides are not restricted to proteins per se that native Mycoplasma gallisepticum expresses, and may include modified polypeptides. For example, one or more amino acids of the polypeptides may be modified naturally or artificially in a conventional manner such as site-specific mutation, etc. (JPB 6-16709, etc.) through loss, addition, insertion, deletion, substitution, etc. Of course, the proteins, even after such modification, should contain the epitope showing the antigenicity. For determination of the epitope region, there are available known methods based on the peptide scanning technique such as the method of Geysen et al. (J. Immunol. Meth., 102, 259-274 (1987)), the method of Hopp et al., (Proc. Natl. Acad. USA, 78, 3824-3828 (1981)), the method Chou et al. (Advances in Enzymology, 47, 145-148 (1987)), etc.

[0015] Specific examples of the peptides having the antigenicity include antigenic proteins disclosed in JPA 2-111795 (U.S. Patent Application Serial Nos. 359,779, 07/888,320 and 08/299,662), JPA 5-824646 (U.S. Patent No. 5,489,430), WO 94/23019 (U.S. Patent Application Serial No. 08/525,742, JPA 6-521927) and proteins of Mycoplasma gallisepticum containing the amino acid sequences of those proteins. Of course, so long as the epitope is contained therein, a part of the peptides described above may also be usable.

[0016] Of these peptides, preferred are the polypeptide of about 40 killodaltons (kd) described in JPA 5-824646, the polypeptide of about 66 kd encoded by TM-66 gene and the polypeptide of about 67 kd encoded by TM-67 gene described in JPA 5-521927, which are designated as SEQ NO: 16 and SEQ NO: 27 therein.

[0017] In the present invention, genes of the Mycoplasma-derived polypeptides bear DNA sequences coding for the polypeptide having the antigenicity of Mycoplasma gallisepticum described above. Such DNA can be obtained by synthesis or acquired from wild bacteria belonging to Mycoplasma gallisepticum. Specific examples of such bacteria are strains R, S6, KP-13, PG31, etc. DNA may also be derived from MG isolated from wild strains. These genes can also be modified by loss, addition, insertion, deletion, substitution, etc. in a conventional manner as described in Methods in Enzymology, etc.

Herpesvirus-derived polypeptides and genes thereof

[0018] The Herpesvirus-derived polypeptides in the present invention refer to polypeptides derived from proteins that

construct an envelope of viruses belonging to the genus Herpesvirus. The Herpesvirus-derived polypeptides may not always be the full length of the proteins. Where the polypeptides are used solely to be expressed on the surface of cell membranes function as fusion proteins, it is sufficient for the polypeptide to contain a membrane anchor and a signal sequence therein, and where the polypeptides are employed for secretion, the polypeptides may contain only a signal sequence for that purpose. The outer membrane proteins may be either type I or type II of the outer membrane proteins. The signal sequence and the membrane anchoring sequence are both readily detectable by analyzing the amino acid sequence in the hydrophobic peptide region at the carboxyl terminus or amino terminus thereof.

[0019] Specific examples of the outer membrane protein include gB, gC, gD, gH and gI which are glycoproteins of herpes simplex viruses, and gBh, gCh, gDh, gHh and gIh of MDV corresponding to herpes simplex viruses glycoproteins gB, gC, gD, gH and gI, and proteins of the genus Herpesvirus homologous to the proteins described above.

[0020] Of course, polypeptides bearing the epitope other than the signal sequence of the outer membrane proteins may also be ligated with the aforesaid polypeptides having the antigenicity. By the ligation it is expected that the epitope will give the immunity to the living body in vivo.

[0021] In the present invention, the genes for the Mycoplasma-derived polypeptides contain DNA sequences coding for the Herpesvirus-derived polypeptides described above and such DNAs can be synthesized or acquired from naturally occurring herpes viruses. These genes may also be modified by loss, addition, insertion, deletion, substitution, etc. in a conventional manner as described in Methods in Enzymology, etc.

Fusion protein and hybrid DNA

[0022] The fusion proteins of the present invention are obtained by incubating a recombinant Avipox virus inserted hydrid DNA, which will be later described, in culture cells such as chick embryo fibroblast cells (hereinafter referred to as CEF cells) or embryonated chorioallantoic membrane cells, etc.

[0023] The thus obtained fusion proteins can be employed as a component vaccine.

[0024] The hybrid DNA of the present invention comprises the gene for the Mycoplasma-derived polypeptide and the gene for the Herpesvirus-derived polypeptide, which are ligated with each other directly or via an optional DNA sequence.

[0025] The hybrid DNA of the present invention can be produced in a conventional manner, for example, by a method in which the outer membrane protein and the antigenic protein of Mycoplasma gallisepticum are digested with restriction enzymes, respectively, and the resulting ligatable DNA fragment coding for the outer membrane protein of herpes viruses or for the signal sequence of the outer membrane protein is ligated with the resulting ligatable DNA fragment coding for the antigenic protein of Mycoplasma gallisepticum, using a ligase directly or via an appropriate linker.

[0026] Specific examples of the amino acid sequences for the fusion proteins of the present invention include SEQ NO: 2 and SEQ NO: 4. The sequence of the antigenic protein of 40 killodaltons derived from Mycoplasma gallisepticum is found in amino acids 64-456 of SEQ NO: 2 and in amino acids 693-1086 of SEQ NO: 4. The signal sequence of outer membrane protein gB derived from MDV is found in amino acids 1-63 of SEQ NO: 2. In SEQ NO: 4, amino acids 1-672 correspond to almost the full length of outer membrane protein gB derived from MDV. Specific examples of nucleotide sequences of the hybrid DNAs coding for these fusion proteins are those shown by SEQ NO: 1 and SEQ NO: 3.

[0027] These fusion proteins and hybrid DNAs are given by way of examples but are not deemed to be limited thereto.

Recombinant Avipox virus

[0028] The recombinant Avipox virus of the present invention is a recombinant Avipox virus in which the aforesaid DNA or hybrid DNA has been inserted in the non-essential region. The recombinant Avipox virus of the present invention can be constructed in a conventional manner, e.g., by the method described in Japanese Patent Application Laid-Open No. 1-168279. That is, the non-essential region of Avipox virus is incorporated into a DNA fragment to construct a first recombinant vector.

[0029] As the non-essential region of Avipox virus which is used in the present invention, there are a TK gene region of quail pox virus, a TK region of turkey pox virus and DNA fragments described in JPA 1-168279, preferably a region which causes homologous recombination with EcoRI fragment of about 7.3 Kb, HindIII fragment of about 5.2 Kb, EcoRI-HindIII fragment of about 5.0 Kb, BamHI fragment of about 4.0 Kb, described in the patent specification supra.

[0030] Examples of the vector used in the present invention include plasmids such as pBR322, pBR325, pBR327, pBR328, pUC7, pUC8, pUC9, pUC18, pUC19, and the like; phages such as λ phage, M13 phage, etc.; cosmid such as pHC79 and the like.

[0031] The Avipox virus used in the present invention is not particularly limited so long as it is a virus infected to avian. Specific examples of such a virus include pigeon pox virus, fowl pox virus (hereafter abbreviated as FPV), canary pox virus, turkey pox virus, preferably pigeon pox virus, FPV and turkey pox virus, more preferably pigeon pox virus and FPV. Specific examples of the most preferred Avipox virus include FPVs such as ATCC VR-251, ATCC VR-249, ATCC

VR-250, ATCC VR-229, ATCC VR-288, Nishigahara strain, Shisui strain, CEVA strain and a viral strain among CEVA strain-derived viruses which forms a large plaque when infected to chick embryo fibroblast, and a virus such as NP strain (chick embryo-attenuated pigeon pox virus Nakano strain), etc. which is akin to FPV and used as a fowlpox live vaccine strain. These strains are commercially available and readily accessible.

[0032] Next, the hybrid DNA of the present invention is inserted into the non-essential region of the first recombinant vector described above to construct a second recombinant vector. In general, the hybrid DNA employed may have any nucleotide sequence, irrespective of synthetic or natural one, so long as the hybrid DNA effectively functions as a promoter in the system of transcription possessed by Avipox viruses. Accordingly, not only promoters inherent to Avipox viruses such as promoters for Avipox virus-derived genes coding for thymidine kinase but also DNAs derived from viruses other than Avipox viruses and DNAs derived from eukaryotes or prokaryotes may also be employed in the present invention, insofar as these substances meet the requirements described above. Specific examples of such promoters include promoters for vaccinia viruses (hereinafter often referred to as VV) as described in Journal of Virology, 51, 662-669 (1984), more specifically, a promoter of VV gene coding for 7.5 K polypeptide, a promoter of VV gene coding for 19 K polypeptide, a promoter of VV gene coding for 42 K polypeptide, a promoter of VV gene coding for thymidine kinase, a promoter of VV gene coding for 28 K polypeptide, etc. Furthermore, there may be used a synthetic promoter obtained by modification of the Moss et al. method (J. Mol. Biol., 210, 49-76 and 771-784, 1989), Davidson's synthetic promoter, a promoter obtained by modifying a part of the Davidson's promoter through deletion or change in such a range that the promoter activity is not lost (e.g.,

TTTTTTTTTTTTGGCATATAAATAATAAATACAATAATTAATTACGCGTAAAAA

TTGAAAAACTATTCTAATTTATTGCACTC,

TTTTTTTTTTTTTTTTTTTGGCATATAAATAATAAATACAATAATTAATTACGCGT

AAAAATTGAAAAACTATTCTAATTTATTGCACTC etc.).

[0033] Further in view of easy detection of the recombinant virus, a marker gene such as a DNA coding for β-galactosidase may also be inserted.

[0034] The recombinant Avipox virus can be constructed by transfecting the second recombinant vector described above to animal culture cells, which has been previously infected with Avipox virus, and causing homologous recombination between the vector DNA and the viral genome DNA. The animal culture cells used herein can be any cells, so long as Avipox can grow in the cells. Specific examples of such animal culture cells are CEF cells, embryonated egg chorioallantoic membrane cells, and the like.

[0035] The objective recombinant Avipox virus is isolated from the virus infected to host cells by plaque hybridization, etc.

Live vaccine

[0036] The recombinant virus of the present invention constructed by the method described above can be inoculated to avian as a live vaccine for Mycoplasma gallisepticum infection.

[0037] The live vaccine of the present invention is prepared by, e.g., the following method, though the process is not particularly limited thereto. The recombinant virus of the present invention is infected to cells in which the virus can grow (hereafter referred to as host cells). After the recombinant virus grows, the cells are recovered and homogenated. The homogenate is centrifuged to separate into the precipitates and the high titer supernatant containing the recombinant virus. The resulting supernatant is substantially free of host cells but contains the cell culture medium and the recombinant virus and hence can be used as a live vaccine. The supernatant may be diluted by adding a pharmacologically inert carrier, e.g., physiological saline, etc. The supernatant may be freeze-dried to be provided for use as a live vaccine. A method for administration of the live vaccine of the present invention to fowl is not particularly limited and examples of the administration include a method for scratching the skin and inoculating the live vaccine on the scratch, effecting the inoculation through injection, oral administration by mixing the live vaccine with feed or drinking water, inhalation by aerosol or spray, etc. In order to use as the live vaccine, the dosage may be the same as ordinary live vaccine; for example, approximately 10^2 to 10^8 plaque forming unit (hereinafter abbreviated as PFU) is inoculated per chick. Where the inoculation is effected by injection, the recombinant virus of the present invention is generally suspended in about 0.1 ml of an isotonic solvent such as physiological saline and the resulting suspension is provided for use. The live vaccine of the present invention can be lyophilized under ordinary conditions and can be stored at room temperature. It is also

possible to freeze the virus suspension at -20 to -70°C and store the frozen suspension.

[0038] Particularly where the genes coding for the polypeptides derived from the outer membrane proteins of herpes viruses described above are those coding for polypeptides having more than one epitope of herpes viruses, preferably having at least 90% homology to native outer membrane proteins, the live vaccine of the present invention functions as a vaccine for both Mycoplasma gallisepticum infection and Avipox viral infection. In addition, the live vaccine of the present invention can also function as an effective vaccine for infection with herpes virus originating from outer membrane proteins. That is, the live vaccine of the present invention can be used as a so-called trivalent vaccine.

EXAMPLES

Example 1

Construction of recombinant pNZ40K-S bearing hybrid DNA ligating TTM-1 protein DNA immediately after the signal of gB gene for Marek's disease virus (cf. Figs. 1, 2 and 3)

[0039] First, plasmid pUCgB bearing gB gene of Marek's disease virus, disclosed in JPA 6-78764, was digested with restriction enzymes BamHI and SalI to recover a fragment of 3.9 kb.

[0040] Separately, plasmid pGTPs was constructed by digesting plasmid pNZ1729R (Yanagida et al., J. Virol., 66, 1402-1408 (1992)) with HindIII and SalI, inserting the resulting DNA fragment of about 140 bp into pUC18 at the HindIII-SalI site thereof, further inserting synthetic DNA (5'-AGCTGCCCCCCCGGCAAGCTTGCA-3') at the HindIII-PstI site, then inserting synthetic DNA (5'-TCGACATTTTTATGTGTAC-3') at the SalI-EcoRI site and finally inserting synthetic DNA (5'-AATCGGCCGGGGGGGCCAGCT-3') at the SacI-EcoRI site.

[0041] The thus obtained pGTPs was digested with restriction enzymes SalI and BamHI and then ligated with the aforesaid 3.9 kb fragment using a ligase to obtain pGTPsMDgB. Thereafter, pNZ2929XM1 disclosed in WO 94/23019 was digested with EcoRI to recover a fragment of 740 bp and then obtained a blunt end with T4 DNA polymerase. On the other hand, pGTPsMDgB was also digested with XbaI and then obtained a blunt end with T4 DNA polymerase. Subsequently, pGTPsMDgB was ligated with the 740 bp fragment having the blunt end using a ligase to construct a new plasmid. This new plasmid was digested with BglII and SalI to recover a fragment of 3.0 kb. The 3.0 kb fragment was ligated with the 1.1 kb fragment obtained through digestion of pNZ2927XM1 with BglII and SalI, using a ligase. Thus, there was obtained a plasmid ligating the N terminus of TTM-1 gene at the C terminus of the signal sequence of gB gene of Marek's disease virus.

[0042] Finally, a fragment of 1.4 kb obtained by digestion of pGTPs40K-S with SalI and BamHI was ligated with a fragment of 9.3 kb obtained by digestion of plasmid pNZ1829R with SalI and BamHI, using a ligase. The objective plasmid pNZ40K-S of 10.7 kb was thus constructed for use in recombination.

Example 2

Construction of recombinant pNZ40K-C bearing hybrid DNA ligating TTM-1 protein DNA at the C terminus of gB gene for Marek's disease virus (cf. Figs. 4, 5 and 6)

[0043] After plasmid pGTPsMDgB obtained in Example 1 was digested with restriction enzyme MluI, and then obtained a blunt end with T4 DNA polymerase, which was followed by digestion with restriction enzyme XbaI to recover a fragment of 1.9 kb. Separately, pBluescriptII (made by Toyobo Co., Ltd., hereinafter abbreviated as pBSKSII) was digested with restriction enzymes XbaI and SmaI. The resulting fragment was ligated with the 1.9 kb fragment obtained above using a ligase to give a plasmid. The resulting plasmid was digested with restriction enzymes EcoRI and SalI. The resulting fragment was ligated with the 550 bp fragment and the 615 bp fragment, both obtained by digestion of pNZ2929XM1 with restriction enzymes EcoRI and SalI and with restriction enzymes EcoT22I and SalI, respectively, using a ligase to construct a plasmid. The thus obtained plasmid was digested with restriction enzymes XbaI and SalI. The resulting 2.7 kb fragment was ligated with the 3.3 kb fragment obtained by digestion of pGTPsMDgB with restriction enzymes XbaI and SalI, using a ligase. Plasmid pGTPs40K-C ligating the TTM-1 gene at the N terminus thereof with the gB gene for Marek's disease virus at the C terminus thereof was thus obtained.

[0044] Finally, a fragment of 2.7 kb obtained by digestion of pGTPs40K-C with SalI and XbaI was ligated with a fragment of 9.5 kb obtained by digestion of plasmid pNZ1829R with SalI and XbaI, using a ligase. The objective plasmid pNZ40K-C of 12.2 kb for recombination was thus constructed.

Example 3

Construction of recombinants FPV 40K-C and 40K-S and purification thereof

[0045] NP strain, which is a fowlpox live vaccine strain, was infected to monolayered CEF at m.o.i. = 0.1. Three hours after, these cells were scraped off from the monolayer by a treatment with trypsin to form a cell suspension. After 2 x 10^7 cells in the suspension were mixed with 10 μg of plasmid pNZ40K-C or pNZ40K-S for use in recombination, the mixture was suspended in Saline G (0.14 M sodium chloride, 0.5 mM potassium chloride, 1.1 mM disodium hydrogenphosphate, 1.5 mM potassium dihydrogenphosphate, 0.5 mM magnesium chloride hexahydrate, 0.011% glucose). The suspension was subjected to electrophoresis under conditions of 3.0 kV cm^{-1}, 0.4 msec and 25°C, using Gene Pulser (manufactured by Bio-Rad Co., Ltd.) at room temperature. The plasmid-infected cells were then cultured at 37°C for 72 hours. The cells were lysed by freezing and thawing 3 times to recover viruses containing the recombinant virus.

[0046] The recovered recombinant virus was selected as follows. The recovered viral solution was infected to monolayered CEF and 10 ml of agar solution containing growth medium was overlaid thereon. After agar was warmed at room temperature, incubation was performed at 37°C until plaques of FPV appeared. Then agar medium containing Bluo-gal in a concentration of 200 μg/ml was overlaid on the agar followed by incubation at 37°C for further 48 hours. Among all of the plaques, about 1% of the plaques were colored blue. These blue plaques were isolated and recovered. By the same procedures, isolation and recovery were repeated to purify the virus until all the plaques were stained to blue. In general, the repeated procedures were terminated by 3 to 4 days. The purified strains were named 40K-C and 40K-S, respectively. In 40K-C and 40K-S, each position of the DNAs inserted was confirmed by dot blotting hybridization and Southern blotting hybridization.

Example 4

Expression of TTM-1 polypeptide in cells infected with 40K-C and 40K-S

[0047] In order to confirm that 40K-C and 40K-S could express TTM-1 polypeptide in infected cells, Western blotting was performed using anti-*Mycoplasma gallisepticum* S6 strain sera. Virus 40K-C or 40K-S was infected to CEF and cultured at 37°C until plaques were formed. The cells were then scraped off with a cell scraper and centrifuged at 8000G for 20 minutes together with the culture supernatant. The cell-containing precipitates (hereinafter referred to as pellets) were recovered. After washing with PBS, the pellets were centrifuged at 8000G for 20 minutes followed by rinsing to recover the pellets. The pellets were then suspended in 150 μl of PBS. From the suspension 50 μl was taken and added with the same volume of Laemmli's buffer (containing 10% mercapto-ethanol). After boiling for 3 minutes, the mixture was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (hereinafter abbreviated as SDS-PAGE) in accordance with the Laemmli's method (Nature, 227, 668-685 (1970)). The polypeptides isolated on the SDS-PAGE-completed gel were transferred onto a polyvinylidene difluoride membrane (Immobilon Transfer Membrane, made by Millipore Inc., hereinafter simply referred to as membrane) according to the method of Burnett et al., (A. Anal. Biochem., 112, 195-203 (1970)) or by the method of Towbin et al. (Proc. Natl. Acad. Sci., 75, 4350-4354 (1979)) by means of electrophoresis. The membrane was dipped for an hour into PBS containing 3% skimmed milk for blocking not to cause any non-specific binding. Next, the membrane was dipped for an hour in PBS in which chick anti-*Mycoplasma gallisepticum* S6 strain serum was diluted to 1000-fold.

[0048] Subsequently, the membrane was rinsed with PBS and then dipped for an hour in PBS containing alkaline phosphatase conjugate anti-chick IgG as a secondary antibody. After the membrane was rinsed with PBS, a color-forming reaction was carried out in 10 ml of a solution containing 100 mM Tris hydrochloride (pH 7.5), 0.15 M sodium chloride and 50 mM magnesium chloride, using Nitro Blue Tetrazolium salt (NBT, made by GIBCO-BRL Inc.) and 5-bromo-4-chloro-3-indole phosphate-p-toluidine (BCIP, made by GIBCO-BRL Inc.) as color-forming substrates.

[0049] The results of the Western blotting are shown in Fig. 7.

[0050] As shown in Fig. 7, proteins could be confirmed with the cells infected both with 40K-S and 40K-C as those reactive at the objective positions. It was thus verified that the expected proteins could be expressed in the recombinant FPV infected cells.

Example 5

Antibody-inducing capability of recombinant FPV-inoculated chicken

[0051] After 40K-C and 40K-S were cultured in CEF at 37°C for 48 hours, the procedure of freezing and thawing was repeated twice to recover the cell suspension. The cell suspension was adjusted to have a virus titer of 10^6 pfu/ml and then inoculated to SPF chicken (Line M, Nippon Seibutsu Kagaku Kenkyusho) of 7 days old at the right wing web in a

dose of 10 μl through a stab needle. After the inoculation, take of the pock was observed and the sera were collected 2 weeks after the inoculation. The antibody titer of the sera collected was determined by ELISA. The purified TTM-1 polypeptide was dissolved in a bicarbonate buffer solution in a concentration of 1 μg/well. After adsorption to a 96 well microtiter plate, blocking was effected with skimmed milk to prevent the subsequent non-specific adsorption. Next, a dilution of the sample serum was charged in each well and then horse radish peroxide-labeled anti-chicken immunoglobulin antibody (rabbit antibody) was added thereto as a secondary antibody. After thoroughly washing, 2,2'-azinodiethylbenzothiazoline sulfonate was added to the mixture as a substrate and a relative dilution magnification of the antibody was measured with an immuno-reader in terms of absorbance at a wavelength of 405 nm. As a primary antibody for control, anti-TTM-1 polypeptide chicken serum was used. The results are shown in Table 1.

Table 1

Antibody titer of rFPV-inoculated chicken by ELISA	
Methods for treating chicken	Antibody titer of anti-TTM-1 polypeptide
40K-S inoculation	1024
40K-C inoculation	512
TTM-1 immunization	512
non-inoculated	1
Antibody titer: Dilution magnification when the antibody titer of the group of non-inoculated chicken serum dilution was made 1	

[0052] As shown in Table 1, the results reveal that when 40K-C or 40K-S was inoculated to chicken, the anti-TTM-1 antibody titer in sera was increased to the level higher than the antibody titer in sera from the chicken immunized with TTM-1 polypeptide. From the results it was confirmed that the recombinant FPV could significantly induce the antibody titer to the inoculated chicken.

Example 6

Mycoplasma challenge test against recombinant FPV-inoculated chicken

[0053] The challenge test was conducted basically in accordance with the standard for biological preparations for animals. The method is briefly described below.

[0054] Strains 40K-C and 40K-S were inoculated to SPF chicken (Line M, Japan Biological Science Laboratory) of 5 weeks old at the right wing web in a dose of 10 μl through a stab needle. After the inoculation, take of the pock was observed to verify completion of the immunization. Two weeks after the inoculation, *Mycoplasma gallisepticum* strain R was forced to be intratracheally administered in a dose of 10^4 to 10^5 cfu/chick, whereby infection was made sure. On Day 14 after the infection, the chicken were enthanized with Nembutal. Tissue sections were prepared from the tracheal lesion and scores of the tracheal lesion were determined based on the thickness of tracheal mucous membrane and histological findings. The scores were also determined by the above standard for biological preparations. An average of scores for the tracheal lesion observed with each chick in the groups was made the score for the respective groups. For information, criteria to determine tracheal lesion scores is shown in Table 2.

Table 2. Standard Criteria for Scoring Tracheal Lesion

Thickness of Mucous Membrane	Histological Finding	Score
90 µm -	normal appearance of ciliated epitherial cells and mucus gland	0
90 µm - 110 µm	In the lamina propria, slight infiltration of round cells or minute nest can be found, but epithelial cell-layer is normal.	1
110 µm -	Epitherial cell are degenarated or diseminated, and the lamina propria is moderately thickened due to round cells infiltration.	2
	Squamous metaplasia of surface epithelium and lamina propria is extremely thickened due to capillary hyperplasia and rounded cells infiltration; cell debris are accumulated in the tracheal lumen.	3

[0055] The results of evaluation are shown in Table 3 and Fig. 8.

Table 3

Means tracheal lesion scores in FPV-inoculated Chicken		
Vaccination	Lesion Score	
	Average	Standard Error
40K-S	1.38	0.16

Table 3 (continued)

Means tracheal lesion scores in FPV-inoculated Chicken		
Vaccination	Lesion Score	
	Average	Standard Error
40K-C	1.89	0.13
Commercial vaccine	2.11	0.24
TTM-1 polypeptide	1.09	0.23
None	2.27	0.21

[0056] As is clearly noted from the results above, the lesion scores of chicken inoculated with 40K-C and 40K-S are obviously low as compared to that of the non-inoculated chicken, indicating that the vaccines of the present invention clearly imparted to chicken the effective infection prevention for Mycoplasma challenge. Thus, the results reveal that 40K-C and 40K-S could be effective vaccines for Mycoplasma gallisepticum.

INDUSTRIAL APPLICABILITY

[0057] According to the present invention, the fusion proteins of the polypeptides derived from antigenic proteins of Mycoplasma gallisepticum and the polypeptides derived from outer membrane proteins of herpes viruses are obtained. The fusion proteins are effective as vaccines for anti-Mycoplasma infection, anti-chicken pox or anti-Marek's disease. By use of the hybrid DNAs coding for the fusion proteins, Mycoplasma gallisepticum antigenic proteins can be efficiently provided on the surface of host cells. Moreover, the hybrid DNAs can secrete the antigenic proteins extracellularly to obtain Avipox viruses that can be efficiently recognized by the antigen recognizing cells in host cells. The thus obtained recombinant Avipox viruses are useful as potent vaccines for anti-Mycoplasma infection.

SEQUENCE LISTING

SEQ NO: 1

Length of sequence: 1371

Type of sequence: nucleic acid

Number of strand: double strand

Topology: linear

Kind of sequence: other nucleic acid, hybrid DNA (40K-S)

Sequence:

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ATG CAC TAT TTT AGG CGG AAT TGC ATA TTT TTC CTT ATA GTT ATT CTA    48
Met His Tyr Phe Arg Arg Asn Cys Ile Phe Phe Leu Ile Val Ile Leu
  1               5                  10                  15
TAT GGT ACG AAC TCA TCT CCG AGT ACC CAA AAT GTG ACA TCA AGA GAA    96
Tyr Gly Thr Asn Ser Ser Pro Ser Thr Gln Asn Val Thr Ser Arg Glu
             20                  25                  30
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GTT GTT TCG AGC GTC CAG TTG TCT GAG GAA GAG TCT ACG TTT TAT CTT 144
Val Val Ser Ser Val Gln Leu Ser Glu Glu Glu Ser Thr Phe Tyr Leu
35 40 45

TGT CCC CCA CCA GTG GGT TCA ACC GTG ATC CGT CTA GAA TTC GGC TGT 192
Cys Pro Pro Pro Val Gly Ser Thr Val Ile Arg Leu Glu Phe Gly Cys
50 55 60

ATG TCT ATT ACT AAA AAA GAT GCA AAC CCA AAT AAT GGC CAA ACC CAA 240
Met Ser Ile Thr Lys Lys Asp Ala Asn Pro Asn Asn Gly Gln Thr Gln
65 70 75 80

TTA GAA GCA GCG CGA ATG GAG TTA ACA GAT CTA ATC AAT GCT AAA GCG 288
Leu Glu Ala Ala Arg Met Glu Leu Thr Asp Leu Ile Asn Ala Lys Ala
85 90 95

ATG ACA TTA GCT TCA CTA CAA GAC TAT GCC AAG ATT GAA GCT AGT TTA 336
Met Thr Leu Ala Ser Leu Gln Asp Tyr Ala Lys Ile Glu Ala Ser Leu
100 105 110

TCA TCT GCT TAT AGT GAA GCT GAA ACA GTT AAC AAT AAC CTT AAT GCA 384
Ser Ser Ala Tyr Ser Glu Ala Glu Thr Val Asn Asn Asn Leu Asn Ala
115 120 125

ACA TTA GAA CAA CTA AAA ATG GCT AAA ACT AAT TTA GAA TCA GCC ATC 432
Thr Leu Glu Gln Leu Lys Met Ala Lys Thr Asn Leu Glu Ser Ala Ile
130 135 140

AAC CAA GCT AAT ACG GAT AAA ACG ACT TTT GAT AAT GAA CAC CCA AAT 480
Asn Gln Ala Asn Thr Asp Lys Thr Thr Phe Asp Asn Glu His Pro Asn
145 150 155 160

TTA GTT GAA GCA TAC AAA GCA CTA AAA ACC ACT TTA GAA CAA CGT GCT 528
Leu Val Glu Ala Tyr Lys Ala Leu Lys Thr Thr Leu Glu Gln Arg Ala
165 170 175

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ACT AAC CTT GAA GCT TTG TCA TCA ACT GCT TAT AAT CAA ATT CGC AAT    576
Thr Asn Leu Glu Gly Leu Ser Ser Thr Ala Tyr Asn Gln Ile Arg Asn
            180                 185                 190
AAT TTA GTG GAT CTA TAC AAT AAA GCT AGT AGT TTA ATA ACT AAA ACA    624
Asn Leu Val Asp Leu Tyr Asn Lys Ala Ser Ser Leu Ile Thr Lys Thr
        195                 200                 205
CTA GAT CCA CTA AAT GGG GGA ACG CTT TTA GAT TCT AAT GAG ATT ACT    672
Leu Asp Pro Leu Asn Gly Gly Thr Leu Leu Asp Ser Asn Glu Ile Thr
    210                 215                 220
ACA GCT AAT AAG AAT ATT AAT AAT ACG TTA TCA ACT ATT AAT GAA CAA    720
Thr Ala Asn Lys Asn Ile Asn Asn Thr Leu Ser Thr Ile Asn Glu Gln
225                 230                 235                 240
AAG ACT AAT GCT GAT GCA TTA TCT AAT AGT TTT ATT AAA AAA GTG ATT    768
Lys Thr Asn Ala Asp Ala Leu Ser Asn Ser Phe Ile Lys Lys Val Ile
                245                 250                 255
CAA AAT AAT GAA CAA AGT TTT GTA GGG ACT TTT ACA AAC GCT AAT GTT    816
Gln Asn Asn Glu Gln Ser Phe Val Gly Thr Phe Thr Asn Ala Asn Val
            260                 265                 270
CAA CCT TCA AAC TAC AGT TTT GTT GCT TTT AGT GCT GAT GTA ACA CCC    864
Gln Pro Ser Asn Tyr Ser Phe Val Ala Phe Ser Ala Asp Val Thr Pro
        275                 280                 285
GTC AAT TAT AAA TAT GCA AGA AGG ACC GTT TGG AAT GGT GAT GAA CCT    912
Val Asn Tyr Lys Tyr Ala Arg Arg Thr Val Trp Asn Gly Asp Glu Pro
    290                 295                 300
TCA AGT AGA ATT CTT GCA AAC ACG AAT AGT ATC ACA GAT GTT TCT TGG    960
Ser Ser Arg Ile Leu Ala Asn Thr Asn Ser Ile Thr Asp Val Ser Trp
305                 310                 315                 320
```

ATT TAT AGT TTA GCT GGA ACA AAC ACG AAG TAC CAA TTT AGT TTT AGC 1008
Ile Tyr Ser Leu Ala Gly Thr Asn Thr Lys Tyr Gln Phe Ser Phe Ser
325 330 335

AAC TAT GGT CCA TCA ACT GGT TAT TTA TAT TTC CCT TAT AAG TTG GTT 1056
Asn Tyr Gly Pro Ser Thr Gly Tyr Leu Tyr Phe Pro Tyr Lys Leu Val
340 345 350

AAA GCA GCT GAT GCT AAT AAC GTT GGA TTA CAA TAC AAA TTA AAT AAT 1104
Lys Ala Ala Asp Ala Asn Asn Val Gly Leu Gln Tyr Lys Leu Asn Asn
355 360 365

GGA AAT GTT CAA CAA GTT GAG TTT GCC ACT TCA ACT AGT GCA AAT AAT 1152
Gly Asn Val Gln Gln Val Glu Phe Ala Thr Ser Thr Ser Ala Asn Asn
370 375 380

ACT ACA GCT AAT CCA ACT CCA GCA GTT GAT GAG ATT AAA GTT GCT AAA 1200
Thr Thr Ala Asn Pro Thr Pro Ala Val Asp Glu Ile Lys Val Ala Lys
385 390 395 400

ATC GTT TTA TCA GGT TTA AGA TTT GGC CAA AAC ACA ATC GAA TTA AGT 1248
Ile Val Leu Ser Gly Leu Arg Phe Gly Gln Asn Thr Ile Glu Leu Ser
405 410 415

GTT CCA ACG GGT GAA GGA AAT ATG AAT AAA GTT GCG CCA ATG ATT GGC 1296
Val Pro Thr Gly Glu Gly Asn Met Asn Lys Val Ala Pro Met Ile Gly
420 425 430

AAC ATT TAT CTT AGC TCA AAT GAA AAT AAT GCT GAT AAG ATC CCC GGG 1344
Asn Ile Tyr Leu Ser Ser Asn Glu Asn Asn Ala Asp Lys Ile Pro Gly
435 440 445

TAC CGT CGA CCC GGT ACA TTT TTA TAA 1371
Tyr Arg Arg Pro Gly Thr Phe Leu ***
450 455

SEQUENCE LISTING

SEQ NO: 2

Length of sequence: 456

Type of sequence: amino acid

Topology: linear

Kind of sequence: protein

Sequence:

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Met His Tyr Phe Arg Arg Asn Cys Ile Phe Phe Leu Ile Val Ile Leu
  1               5                  10                  15
Tyr Gly Thr Asn Ser Ser Pro Ser Thr Gln Asn Val Thr Ser Arg Glu
            20                  25                  30
Val Val Ser Ser Val Gln Leu Ser Glu Glu Glu Ser Thr Phe Tyr Leu
        35                  40                  45
Cys Pro Pro Pro Val Gly Ser Thr Val Ile Arg Leu Glu Phe Gly Cys
    50                  55                  60
Met Ser Ile Thr Lys Lys Asp Ala Asn Pro Asn Asn Gly Gln Thr Gln
65                  70                  75                  80
Leu Glu Ala Ala Arg Met Glu Leu Thr Asp Leu Ile Asn Ala Lys Ala
                85                  90                  95
Met Thr Leu Ala Ser Leu Gln Asp Tyr Ala Lys Ile Glu Ala Ser Leu
            100                 105                 110
Ser Ser Ala Tyr Ser Glu Ala Glu Thr Val Asn Asn Asn Leu Asn Ala
        115                 120                 125
Thr Leu Glu Gln Leu Lys Met Ala Lys Thr Asn Leu Glu Ser Ala Ile
    130                 135                 140
Asn Gln Ala Asn Thr Asp Lys Thr Thr Phe Asp Asn Glu His Pro Asn
145                 150                 155                 160
```

Leu Val Glu Ala Tyr Lys Ala Leu Lys Thr Thr Leu Glu Gln Arg Ala
165 170 175
Thr Asn Leu Glu Gly Leu Ser Ser Thr Ala Tyr Asn Gln Ile Arg Asn
180 185 190
Asn Leu Val Asp Leu Tyr Asn Lys Ala Ser Ser Leu Ile Thr Lys Thr
195 200 205
Leu Asp Pro Leu Asn Gly Gly Thr Leu Leu Asp Ser Asn Glu Ile Thr
210 215 220
Thr Ala Asn Lys Asn Ile Asn Asn Thr Leu Ser Thr Ile Asn Glu Gln
225 230 235 240
Lys Thr Asn Ala Asp Ala Leu Ser Asn Ser Phe Ile Lys Lys Val Ile
245 250 255
Gln Asn Asn Glu Gln Ser Phe Val Gly Thr Phe Thr Asn Ala Asn Val
260 265 270
Gln Pro Ser Asn Tyr Ser Phe Val Ala Phe Ser Ala Asp Val Thr Pro
275 280 285
Val Asn Tyr Lys Tyr Ala Arg Arg Thr Val Trp Asn Gly Asp Glu Pro
290 295 300
Ser Ser Arg Ile Leu Ala Asn Thr Asn Ser Ile Thr Asp Val Ser Trp
305 310 315 320
Ile Tyr Ser Leu Ala Gly Thr Asn Thr Lys Tyr Gln Phe Ser Phe Ser
325 330 335
Asn Tyr Gly Pro Ser Thr Gly Tyr Leu Tyr Phe Pro Tyr Lys Leu Val
340 345 350
Lys Ala Ala Asp Ala Asn Asn Val Gly Leu Gln Tyr Lys Leu Asn Asn
355 360 365
Gly Asn Val Gln Gln Val Glu Phe Ala Thr Ser Thr Ser Ala Asn Asn
370 375 380

Thr Thr Ala Asn Pro Thr Pro Ala Val Asp Glu Ile Lys Val Ala Lys
385 390 395 400
Ile Val Leu Ser Gly Leu Arg Phe Gly Gln Asn Thr Ile Glu Leu Ser
405 410 415
Val Pro Thr Gly Glu Gly Asn Met Asn Lys Val Ala Pro Met Ile Gly
420 425 430
Asn Ile Tyr Leu Ser Ser Asn Glu Asn Asn Ala Asp Lys Ile Pro Gly
435 440 445
Tyr Arg Arg Pro Gly Thr Phe Leu ***
450 455

SEQUENCE LISTING

SEQ NO: 3

Length of sequence: 3261

Type of sequence: nucleic acid

Number of strand: double strand

Topology: linear

Kind of sequence: other nucleic acid, hybrid DNA (40K-C)

Sequence:

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ATG CAC TAT TTT AGG CGG AAT TGC ATA TTT TTC CTT ATA GTT ATT CTA     48
Met His Tyr Phe Arg Arg Asn Cys Ile Phe Phe Leu Ile Val Ile Leu
  1               5                  10                  15
TAT GGT ACG AAC TCA TCT CCG AGT ACC CAA AAT GTG ACA TCA AGA GAA     96
Tyr Gly Thr Asn Ser Ser Pro Ser Thr Gln Asn Val Thr Ser Arg Glu
             20                  25                  30
GTT GTT TCG AGC GTC CAG TTG TCT GAG GAA GAG TCT ACG TTT TAT CTT    144
Val Val Ser Ser Val Gln Leu Ser Glu Glu Glu Ser Thr Phe Tyr Leu
         35                  40                  45
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TGT CCC CCA CCA GTG GGT TCA ACC GTG ATC CGT CTA GAA CCG CCG CGA 192
Cys Pro Pro Pro Val Gly Ser Thr Val Ile Arg Leu Glu Pro Pro Arg
50 55 60

AAA TGT CCC GAA CCT AGA AAA GCC ACC GAG TGG GGT GAA GGA ATC GCG 240
Lys Cys Pro Glu Pro Arg Lys Ala Thr Glu Trp Gly Glu Gly Ile Ala
65 70 75 80

ATA TTA TTT AAA GAG AAT ATC AGT CCA TAT AAA TTT AAA GTG ACG CTT 288
Ile Leu Phe Lys Glu Asn Ile Ser Pro Tyr Lys Phe Lys Val Thr Leu
85 90 95

TAT TAT AAA AAT ATC ATT CAG ACG ACG ACA TGG ACG GGG ACG ACA TAT 336
Tyr Tyr Lys Asn Ile Ile Gln Thr Thr Thr Trp Thr Gly Thr Thr Tyr
100 105 110

AGA CAG ATC ACT AAT CGA TAT ACA GAT AGG ACG CCC GTT TCC ATT GAA 384
Arg Gln Ile Thr Asn Arg Tyr Thr Asp Arg Thr Pro Val Ser Ile Glu
115 120 125

GAG ATC ACG GAT CTA ATC GAC GGC AAA GGA AGA TGC TCA TCT AAA GCA 432
Glu Ile Thr Asp Leu Ile Asp Gly Lys Gly Arg Cys Ser Ser Lys Ala
130 135 140

AGA TAC CTT AGA AAC AAT GTA TAT GTT GAA GCG TTT GAC AGG GAT GCG 480
Arg Tyr Leu Arg Asn Asn Val Tyr Val Glu Ala Phe Asp Arg Asp Ala
145 150 155 160

GGA GAA AAA CAA GTA CTT CTA AAA CCA TCA AAA TTC AAC ACG CCC GAA 528
Gly Glu Lys Gln Val Leu Leu Lys Pro Ser Lys Phe Asn Thr Pro Glu
165 170 175

TCT AGG GCA TGG CAC ACG ACT AAT GAG ACG TAT ACC GTG TGG GGA TCA 576
Ser Arg Ala Trp His Thr Thr Asn Glu Thr Tyr Thr Val Trp Gly Ser
180 185 190

CCA TGG ATA TAT CGA ACG GGA ACC TCC GTC AAT TGT ATA GTA GAG GAA 624
Pro Trp Ile Tyr Arg Thr Gly Thr Ser Val Asn Cys Ile Val Glu Glu
195 200 205

ATG GAT GCC CGC TCT GTG TTT CCG TAT TCA TAT TTT GCA ATG GCC AAT 672
Met Asp Ala Arg Ser Val Phe Pro Tyr Ser Tyr Phe Ala Met Ala Asn
210 215 220

GGC GAC ATC GCG AAC ATA TCT CCA TTT TAT GGT CTA TCC CCA CCA GAG 720
Gly Asp Ile Ala Asn Ile Ser Pro Phe Tyr Gly Leu Ser Pro Pro Glu
225 230 235 240

GCT GCC GCA GAA CCC ATG GGA TAT CCC CAG GAT AAT TTC AAA CAA CTA 768
Ala Ala Ala Glu Pro Met Gly Tyr Pro Gln Asp Asn Phe Lys Gln Leu
245 250 255

GAT AGC TAT TTT TCA ATG GAT TTG GAC AAG CGT CGA AAA GCA AGC CTT 816
Asp Ser Tyr Phe Ser Met Asp Leu Asp Lys Arg Arg Lys Ala Ser Leu
260 265 270

CCA GTC AAG CGT AAC TTT CTC ATC ACA TCA CAC TTC ACA GTT GGG TGG 864
Pro Val Lys Arg Asn Phe Leu Ile Thr Ser His Phe Thr Val Gly Trp
275 280 285

GAC TGG GCT CCA AAA ACT ACT CGT GTA TGT TCA ATG ACT AAG TGG AAA 912
Asp Trp Ala Pro Lys Thr Thr Arg Val Cys Ser Met Thr Lys Trp Lys
290 295 300

GAG GTG ACT GAA ATG TTG CGT GCA ACA GTT AAT GGG AGA TAC AGA TTT 960
Glu Val Thr Glu Met Leu Arg Ala Thr Val Asn Gly Arg Tyr Arg Phe
305 310 315 320

ATG GCC CGT GAA CTT TCG GCA ACG TTT ATC AGT AAT ACG ACT GAG TTT 1008
Met Ala Arg Glu Leu Ser Ala Thr Phe Ile Ser Asn Thr Thr Glu Phe
325 330 335

GAT CCA AAT CGC ATC ATA TTA GGA CAA TGT ATT AAA CGC GAG GCA GAA 1056
Asp Pro Asn Arg Ile Ile Leu Gly Gln Cys Ile Lys Arg Glu Ala Glu
340 345 350

GCA GCA ATC GAG CAG ATA TTT AGG ACA AAA TAT AAT GAC AGT CAC GTC 1104
Ala Ala Ile Glu Gln Ile Phe Arg Thr Lys Tyr Asn Asp Ser His Val
355 360 365

AAG GTT GGA CAT GTA CAA TAT TTC TTG GCT CTC GGG GGA TTT ATT GTA 1152
Lys Val Gly His Val Gln Tyr Phe Leu Ala Leu Gly Gly Phe Ile Val
370 375 380

GCA TAT CAG CCT GTT CTA TCC AAA TCC CTG GCT CAT ATG TAC CTC AGA 1200
Ala Tyr Gln Pro Val Leu Ser Lys Ser Leu Ala His Met Tyr Leu Arg
385 390 395 400

GAA TTG ATG AGA GAC AAC AGG ACC GAT GAG ATG CTC GAC CTG GTA AAC 1248
Glu Leu Met Arg Asp Asn Arg Thr Asp Glu Met Leu Asp Leu Val Asn
405 410 415

AAT AAG CAT GCA ATT TAT AAG AAA AAT GCT ACC TCA TTG TCA CGA TTG 1296
Asn Lys His Ala Ile Tyr Lys Lys Asn Ala Thr Ser Leu Ser Arg Leu
420 425 430

CGG CGA GAT ATT CGA AAT GCA CCA AAT AGA AAA ATA ACA TTA GAC GAC 1344
Arg Arg Asp Ile Arg Asn Ala Pro Asn Arg Lys Ile Thr Leu Asp Asp
435 440 445

ACC ACA GCT ATT AAA TCG ACA TCG TCT GTT CAA TTC GCC ATG CTC CAA 1392
Thr Thr Ala Ile Lys Ser Thr Ser Ser Val Gln Phe Ala Met Leu Gln
450 455 460

TTT CTT TAT GAT CAT ATA CAA ACC CAT ATT AAT GAT ATG TTT AGT AGG 1440
Phe Leu Tyr Asp His Ile Gln Thr His Ile Asn Asp Met Phe Ser Arg
465 470 475 480

ATT GCC ACA GCT TGG TGC GAA TTG CAG AAT AGA GAA CTT GTT TTA TGG 1488
Ile Ala Thr Ala Trp Cys Glu Leu Gln Asn Arg Glu Leu Val Leu Trp
485 490 495

CAC GAA GGG ATA AAG ATT AAT CCT AGC GCT ACA GCG AGT GCA ACA TTA 1536
His Glu Gly Ile Lys Ile Asn Pro Ser Ala Thr Ala Ser Ala Thr Leu
500 505 510

GGA AGG AGA GTG GCT GCA AAG ATG TTG GGG GAT GTC GCT GCT GTA TCG 1584
Gly Arg Arg Val Ala Ala Lys Met Leu Gly Asp Val Ala Ala Val Ser
515 520 525

AGC TGC ACT GCT ATA GAT GCG GAA TCC GTC ACT TTG CAA AAT TCT ATG 1632
Ser Cys Thr Ala Ile Asp Ala Glu Ser Val Thr Leu Gln Asn Ser Met
530 535 540

CGA GTT ATC ACA TCC ACT AAT ACA TGT TAT AGC CGA CCA TTG GTT CTA 1680
Arg Val Ile Thr Ser Thr Asn Thr Cys Tyr Ser Arg Pro Leu Val Leu
545 550 555 560

TTT TCA TAT GGA GAA AAC CAA GGA AAC ATA CAG GGA CAA CTC GGT GAA 1728
Phe Ser Tyr Gly Glu Asn Gln Gly Asn Ile Gln Gly Gln Leu Gly Glu
565 570 575

AAC AAC GAG TTG CTT CCA ACG CTA GAG GCT GTA GAG CCA TGC TCG GCT 1776
Asn Asn Glu Leu Leu Pro Thr Leu Glu Ala Val Glu Pro Cys Ser Ala
580 585 590

AAT CAT CGT AGA TAT TTT CTG TTT GGA TCC GGT TAT GCT TTA TTT GAA 1824
Asn His Arg Arg Tyr Phe Leu Phe Gly Ser Gly Tyr Ala Leu Phe Glu
595 600 605

AAC TAT AAT TTT GTT AAG ATG GTA GAC GCT GCC GAT ATA CAG ATT GCT 1872
Asn Tyr Asn Phe Val Lys Met Val Asp Ala Ala Asp Ile Gln Ile Ala
610 615 620

AGC ACA TTT GTC GAG CTT AAT CTA ACC CTG CTA GAA GAT CGG GAA ATT 1920
Ser Thr Phe Val Glu Leu Asn Leu Thr Leu Leu Glu Asp Arg Glu Ile
625 630 635 640

TTG CCT TTA TCC GTT TAC ACA AAA GAA GAG TTG CGT GAT GTT GGT GTA 1968
Leu Pro Leu Ser Val Tyr Thr Lys Glu Glu Leu Arg Asp Val Gly Val
645 650 655

TTG GAT TAT GCA GAA GTA GCT CGC CGC AAT CAA CTA CAT GAA CTT AAA 2016
Leu Asp Tyr Ala Glu Val Ala Arg Arg Asn Gln Leu His Glu Leu Lys
660 665 670

TTT TAT GAC ATA AAC AAA GTA ATA GAA GTG GAT ACA AAT TAC GCG GGG 2064
Phe Tyr Asp Ile Asn Lys Val Ile Glu Val Asp Thr Asn Tyr Ala Gly
675 680 685

CTG CAG GAA TTC GGC TGT ATG TCT ATT ACT AAA AAA GAT GCA AAC CCA 2112
Leu Gln Glu Phe Gly Cys Met Ser Ile Thr Lys Lys Asp Ala Asn Pro
690 695 700

AAT AAT GGC CAA ACC CAA TTA GAA GCA GCG CGA ATG GAG TTA ACA GAT 2160
Asn Asn Gly Gln Thr Gln Leu Glu Ala Ala Arg Met Glu Leu Thr Asp
705 710 715 720

CTA ATC AAT GCT AAA GCG ATG ACA TTA GCT TCA CTA CAA GAC TAT GCC 2208
Leu Ile Asn Ala Lys Ala Met Thr Leu Ala Ser Leu Gln Asp Tyr Ala
725 730 735

AAG ATT GAA GCT AGT TTA TCA TCT GCT TAT AGT GAA GCT GAA ACA GTT 2256
Lys Ile Glu Ala Ser Leu Ser Ser Ala Tyr Ser Glu Ala Glu Thr Val
740 745 750

AAC AAT AAC CTT AAT GCA ACA TTA GAA CAA CTA AAA ATG GCT AAA ACT 2304
Asn Asn Asn Leu Asn Ala Thr Leu Glu Gln Leu Lys Met Ala Lys Thr
755 760 765

AAT TTA GAA TCA GCC ATC AAC CAA GCT AAT ACG GAT AAA ACG ACT TTT 2352
Asn Leu Glu Ser Ala Ile Asn Gln Ala Asn Thr Asp Lys Thr Thr Phe
770 775 780

GAT AAT GAA CAC CCA AAT TTA GTT GAA GCA TAC AAA GCA CTA AAA ACC 2400
Asp Asn Glu His Pro Asn Leu Val Glu Ala Tyr Lys Ala Leu Lys Thr
785 790 795 800

ACT TTA GAA CAA CGT GCT ACT AAC CTT GAA GGT TTG TCA TCA ACT GCT 2448
Thr Leu Glu Gln Arg Ala Thr Asn Leu Glu Gly Leu Ser Ser Thr Ala
805 810 815

TAT AAT CAA ATT CGC AAT AAT TTA GTG GAT CTA TAC AAT AAA GCT AGT 2496
Tyr Asn Gln Ile Arg Asn Asn Leu Val Asp Leu Tyr Asn Lys Ala Ser
820 825 830

AGT TTA ATA ACT AAA ACA CTA GAT CCA CTA AAT GGG GGA ACG CTT TTA 2544
Ser Leu Ile Thr Lys Thr Leu Asp Pro Leu Asn Gly Gly Thr Leu Leu
835 840 845

GAT TCT AAT GAG ATT ACT ACA GCT AAT AAG AAT ATT AAT AAT ACG TTA 2592
Asp Ser Asn Glu Ile Thr Thr Ala Asn Lys Asn Ile Asn Asn Thr Leu
850 855 860

TCA ACT ATT AAT GAA CAA AAG ACT AAT GCT GAT GCA TTA TCT AAT AGT 2640
Ser Thr Ile Asn Glu Gln Lys Thr Asn Ala Asp Ala Leu Ser Asn Ser
865 870 875 880

TTT ATT AAA AAA GTG ATT CAA AAT AAT GAA CAA AGT TTT GTA GGG ACT 2688
Phe Ile Lys Lys Val Ile Gln Asn Asn Glu Gln Ser Phe Val Gly Thr
885 890 895

TTT ACA AAC GCT AAT GTT CAA CCT TCA AAC TAC AGT TTT GTT GCT TTT 2736
Phe Thr Asn Ala Asn Val Gln Pro Ser Asn Tyr Ser Phe Val Ala Phe
900 905 910

AGT GCT GAT GTA ACA CCC GTC AAT TAT AAA TAT GCA AGA AGG ACC GTT 2784
Ser Ala Asp Val Thr Pro Val Asn Tyr Lys Tyr Ala Arg Arg Thr Val
915 920 925

TGG AAT GGT GAT GAA CCT TCA AGT AGA ATT CTT GCA AAC ACG AAT AGT 2832
Trp Asn Gly Asp Glu Pro Ser Ser Arg Ile Leu Ala Asn Thr Asn Ser
930 935 940

ATC ACA GAT GTT TCT TGG ATT TAT AGT TTA GCT GGA ACA AAC ACG AAG 2880
Ile Thr Asp Val Ser Trp Ile Tyr Ser Leu Ala Gly Thr Asn Thr Lys
945 950 955 960

TAC CAA TTT AGT TTT AGC AAC TAT GGT CCA TCA ACT GGT TAT TTA TAT 2928
Tyr Gln Phe Ser Phe Ser Asn Tyr Gly Pro Ser Thr Gly Tyr Leu Tyr
965 970 975

TTC CCT TAT AAG TTG GTT AAA GCA GCT GAT GCT AAT AAC GTT GGA TTA 2976
Phe Pro Tyr Lys Leu Val Lys Ala Ala Asp Ala Asn Asn Val Gly Leu
980 985 990

CAA TAC AAA TTA AAT AAT GGA AAT GTT CAA CAA GTT GAG TTT GCC ACT 3024
Gln Tyr Lys Leu Asn Asn Gly Asn Val Gln Gln Val Glu Phe Ala Thr
995 1000 1005

TCA ACT AGT GCA AAT AAT ACT ACA GCT AAT CCA ACT CCA GCA GTT GAT 3072
Ser Thr Ser Ala Asn Asn Thr Thr Ala Asn Pro Thr Pro Ala Val Asp
1010 1015 1020

GAG ATT AAA GTT GCT AAA ATC GTT TTA TCA GGT TTA AGA TTT GGC CAA 3120
Glu Ile Lys Val Ala Lys Ile Val Leu Ser Gly Leu Arg Phe Gly Gln
1025 1030 1035 1040

AAC ACA ATC GAA TTA AGT GTT CCA ACG GGT GAA GGA AAT ATG AAT AAA 3168
Asn Thr Ile Glu Leu Ser Val Pro Thr Gly Glu Gly Asn Met Asn Lys
1045 1050 1055

GTT GCG CCA ATG ATT GGC AAC ATT TAT CTT AGC TCA AAT GAA AAT AAT 3216
Val Ala Pro Met Ile Gly Asn Ile Tyr Leu Ser Ser Asn Glu Asn Asn
1060 1065 1070
GCT GAT AAG ATC CCC GGG TAC CGT CGA CCC GGT ACA TTT TTA TAA 3261
Ala Asp Lys Ile Pro Gly Tyr Arg Arg Pro Gly Thr Phe Leu ***
1075 1080 1085

SEQUENCE LISTING

SEQ NO: 4

Length of sequence: 1086

Type of sequence: amino acid

Topology: linear

Kind of sequence: protein

Sequence:

Met His Tyr Phe Arg Arg Asn Cys Ile Phe Phe Leu Ile Val Ile Leu
1 5 10 15

Tyr Gly Thr Asn Ser Ser Pro Ser Thr Gln Asn Val Thr Ser Arg Glu
20 25 30

Val Val Ser Ser Val Gln Leu Ser Glu Glu Glu Ser Thr Phe Tyr Leu
35 40 45

Cys Pro Pro Pro Val Gly Ser Thr Val Ile Arg Leu Glu Pro Pro Arg
50 55 60

Lys Cys Pro Glu Pro Arg Lys Ala Thr Glu Trp Gly Glu Gly Ile Ala
65 70 75 80

Ile Leu Phe Lys Glu Asn Ile Ser Pro Tyr Lys Phe Lys Val Thr Leu
85 90 95

Tyr Tyr Lys Asn Ile Ile Gln Thr Thr Thr Trp Thr Gly Thr Thr Tyr
100 105 110

Arg Gln Ile Thr Asn Arg Tyr Thr Asp Arg Thr Pro Val Ser Ile Glu
115 120 125
Glu Ile Thr Asp Leu Ile Asp Gly Lys Gly Arg Cys Ser Ser Lys Ala
130 135 140
Arg Tyr Leu Arg Asn Asn Val Tyr Val Glu Ala Phe Asp Arg Asp Ala
145 150 155 160
Gly Glu Lys Gln Val Leu Leu Lys Pro Ser Lys Phe Asn Thr Pro Glu
165 170 175
Ser Arg Ala Trp His Thr Thr Asn Glu Thr Tyr Thr Val Trp Gly Ser
180 185 190
Pro Trp Ile Tyr Arg Thr Gly Thr Ser Val Asn Cys Ile Val Glu Glu
195 200 205
Met Asp Ala Arg Ser Val Phe Pro Tyr Ser Tyr Phe Ala Met Ala Asn
210 215 220
Gly Asp Ile Ala Asn Ile Ser Pro Phe Tyr Gly Leu Ser Pro Pro Glu
225 230 235 240
Ala Ala Ala Glu Pro Met Gly Tyr Pro Gln Asp Asn Phe Lys Gln Leu
245 250 255
Asp Ser Tyr Phe Ser Met Asp Leu Asp Lys Arg Arg Lys Ala Ser Leu
260 265 270
Pro Val Lys Arg Asn Phe Leu Ile Thr Ser His Phe Thr Val Gly Trp
275 280 285
Asp Trp Ala Pro Lys Thr Thr Arg Val Cys Ser Met Thr Lys Trp Lys
290 295 300
Glu ValThr Glu Met Leu Arg Ala Thr Val Asn Gly Arg Tyr Arg Phe
305 310 315 320
Met Ala Arg Glu Leu Ser Ala Thr Phe Ile Ser Asn Thr Thr Glu Phe
325 330 335

Asp Pro Asn Arg Ile Ile Leu Gly Gln Cys Ile Lys Arg Glu Ala Glu
340 345 350
Ala Ala Ile Glu Gln Ile Phe Arg Thr Lys Tyr Asn Asp Ser His Val
355 360 365
Lys Val Gly His Val Gln Tyr Phe Leu Ala Leu Gly Gly Phe Ile Val
370 375 380
Ala Tyr Gln Pro Val Leu Ser Lys Ser Leu Ala His Met Tyr Leu Arg
385 390 395 400
Glu Leu Met Arg Asp Asn Arg Thr Asp Glu Met Leu Asp Leu Val Asn
405 410 415
Asn Lys His Ala Ile Tyr Lys Lys Asn Ala Thr Ser Leu Ser Arg Leu
420 425 430
Arg Arg Asp Ile Arg Asn Ala Pro Asn Arg Lys Ile Thr Leu Asp Asp
435 440 445
Thr Thr Ala Ile Lys Ser Thr Ser Ser Val Gln Phe Ala Met Leu Gln
450 455 460
Phe Leu Tyr Asp His Ile Gln Thr His Ile Asn Asp Met Phe Ser Arg
465 470 475 480
Ile Ala Thr Ala Trp Cys Glu Leu Gln Asn Arg Glu Leu Val Leu Trp
485 490 495
His Glu Gly Ile Lys Ile Asn Pro Ser Ala Thr Ala Ser Ala Thr Leu
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Gly Arg Arg Val Ala Ala Lys Met Leu Gly Asp Val Ala Ala Val Ser
515 520 525
Ser Cys Thr Ala Ile Asp Ala Glu Ser Val Thr Leu Gln Asn Ser Met
530 535 540
Arg Val Ile Thr Ser Thr Asn Thr Cys Tyr Ser Arg Pro Leu Val Leu
545 550 555 560

Phe Ser Tyr Gly Glu Asn Gln Gly Asn Ile Gln Gly Gln Leu Gly Glu
565 570 575

Asn Asn Glu Leu Leu Pro Thr Leu Glu Ala Val Glu Pro Cys Ser Ala
580 585 590

Asn His Arg Arg Tyr Phe Leu Phe Gly Ser Gly Tyr Ala Leu Phe Glu
595 600 605

Asn Tyr Asn Phe Val Lys Met Val Asp Ala Ala Asp Ile Gln Ile Ala
610 615 620

Ser Thr Phe Val Glu Leu Asn Leu Thr Leu Leu Glu Asp Arg Glu Ile
625 630 635 640

Leu Pro Leu Ser Val Tyr Thr Lys Glu Glu Leu Arg Asp Val Gly Val
645 650 655

Leu Asp Tyr Ala Glu Val Ala Arg Arg Asn Gln Leu His Glu Leu Lys
660 665 670

Phe Tyr Asp Ile Asn Lys Val Ile Glu Val Asp Thr Asn Tyr Ala Gly
675 680 685

Leu Gln Glu Phe Gly Cys Met Ser Ile Thr Lys Lys Asp Ala Asn Pro
690 695 700

Asn Asn Gly Gln Thr Gln Leu Glu Ala Ala Arg Met Glu Leu Thr Asp
705 710 715 720

Leu Ile Asn Ala Lys Ala Met Thr Leu Ala Ser Leu Gln Asp Tyr Ala
725 730 735

Lys Ile Glu Ala Ser Leu Ser Ser Ala Tyr Ser Glu Ala Glu Thr Val
740 745 750

Asn Asn Asn Leu Asn Ala Thr Leu Glu Gln Leu Lys Met Ala Lys Thr
755 760 765

Asn Leu Glu Ser Ala Ile Asn Gln Ala Asn Thr Asp Lys Thr Thr Phe
770 775 780

Asp Asn Glu His Pro Asn Leu Val Glu Ala Tyr Lys Ala Leu Lys Thr
785 790 795 800

Thr Leu Glu Gln Arg Ala Thr Asn Leu Glu Gly Leu Ser Ser Thr Ala
805 810 815

Tyr Asn Gln Ile Arg Asn Asn Leu Val Asp Leu Tyr Asn Lys Ala Ser
820 825 830

Ser Leu Ile Thr Lys Thr Leu Asp Pro Leu Asn Gly Gly Thr Leu Leu
835 840 845

Asp Ser Asn Glu Ile Thr Thr Ala Asn Lys Asn Ile Asn Asn Thr Leu
850 855 860

Ser Thr Ile Asn Glu Gln Lys Thr Asn Ala Asp Ala Leu Ser Asn Ser
865 870 875 880

Phe Ile Lys Lys Val Ile Gln Asn Asn Glu Gln Ser Phe Val Gly Thr
885 890 895

Phe Thr Asn Ala Asn Val Gln Pro Ser Asn Tyr Ser Phe Val Ala Phe
900 905 910

Ser Ala Asp Val Thr Pro Val Asn Tyr Lys Tyr Ala Arg Arg Thr Val
915 920 925

Trp Asn Gly Asp Glu Pro Ser Ser Arg Ile Leu Ala Asn Thr Asn Ser
930 935 940

Ile Thr Asp Val Ser Trp Ile Tyr Ser Leu Ala Gly Thr Asn Thr Lys
945 950 955 960

Tyr Gln Phe Ser Phe Ser Asn Tyr Gly Pro Ser Thr Gly Tyr Leu Tyr
965 970 975

Phe Pro Tyr Lys Leu Val Lys Ala Ala Asp Ala Asn Asn Val Gly Leu
980 985 990

Gln Tyr Lys Leu Asn Asn Gly Asn Val Gln Gln Val Glu Phe Ala Thr
995 1000 1005

Ser Thr Ser Ala Asn Asn Thr Thr Ala Asn Pro Thr Pro Ala Val Asp
1010 1015 1020
Glu Ile Lys Val Ala Lys Ile Val Leu Ser Gly Leu Arg Phe Gly Gln
1025 1030 1035 1040
Asn Thr Ile Glu Leu Ser Val Pro Thr Gly Glu Gly Asn Met Asn Lys
1045 1050 1055
Val Ala Pro Met Ile Gly Asn Ile Tyr Leu Ser Ser Asn Glu Asn Asn
1060 1065 1070
Ala Asp Lys Ile Pro Gly Tyr Arg Arg Pro Gly Thr Phe Leu ***
1075 1080 1085

Claims

1. A fusion protein comprising a polypeptide having the antigenicity of Mycoplasma gallisepticum and a polypeptide derived from Herpesvirus outer membrane protein, said polypeptide derived from the outer membrane protein being ligated with the polypeptide having the antigenicity of Mycoplasma gallisepticum at the N terminus thereof.

2. A fusion protein according to claim 1, wherein said outer membrane protein is derived from a herpes virus showing infection to fowl.

3. A fusion protein according to claim 2, wherein said outer membrane protein is derived from a Marek's disease virus.

4. A fusion protein according to claim 3, wherein said outer membrane protein is gB protein derived from a Marek's disease virus.

5. A fusion protein according to claim 1, wherein said polypeptide derived from the outer membrane protein is a signal sequence portion in the outer membrane protein derived from a herpes virus.

6. A fusion protein according to claim 5, wherein said outer membrane protein is a signal sequence portion in the outer membrane protein derived from a herpes virus showing infection to fowl.

7. A fusion protein according to claim 5, wherein said signal sequence portion is a signal sequence portion in derived from the outer membrane protein of a Marek's disease virus.

8. A fusion protein according to claim 5, wherein said polypeptide derived from the outer membrane protein is a signal sequence portion of gB protein derived from a Marek's disease virus.

9. A hybrid DNA coding for the fusion protein according to any one of claims 1 through 8.

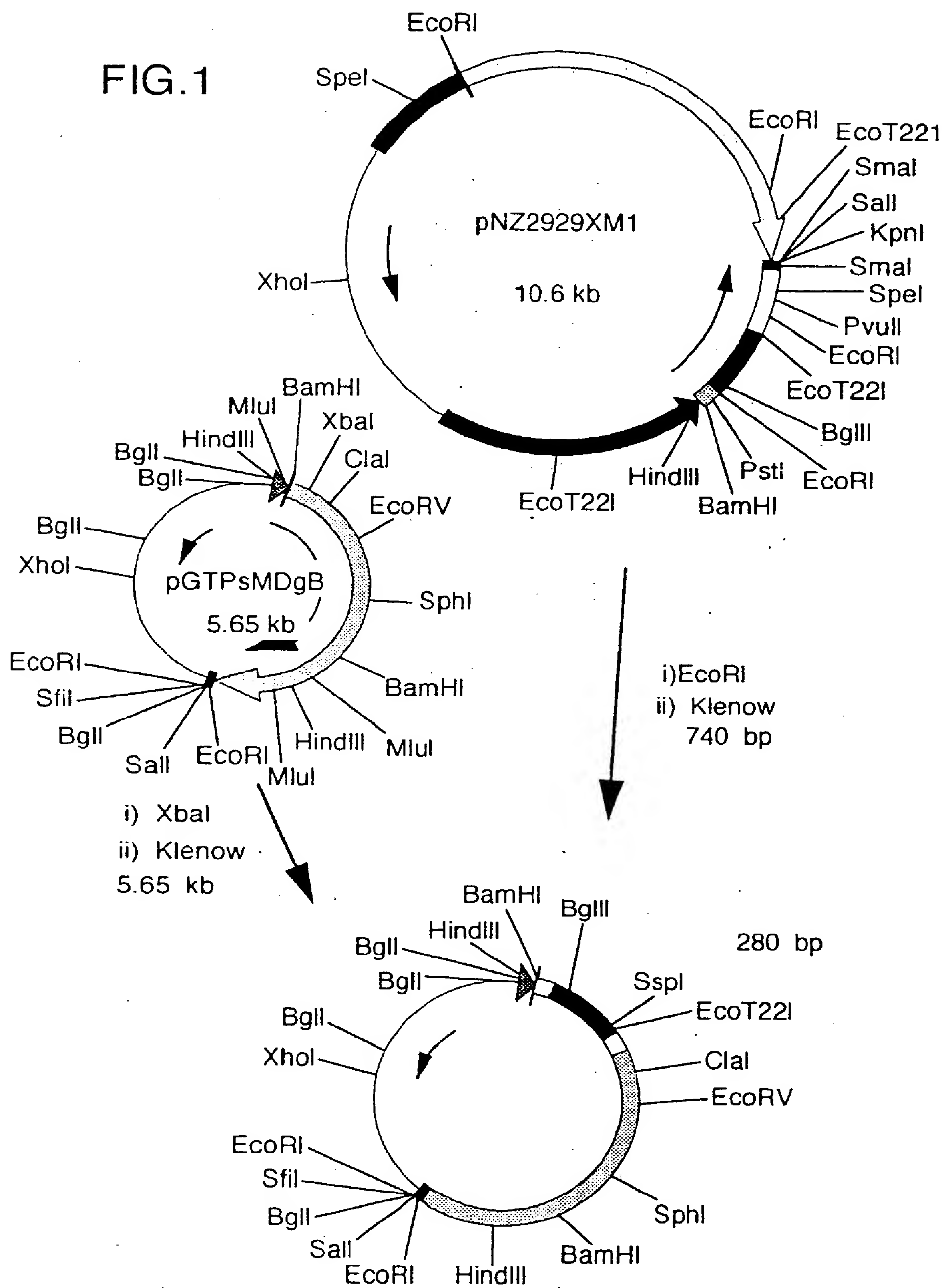
10. A recombinant vector in which a DNA coding for the fusion protein according to any one of claims 1 through 8 has been inserted.

11. A recombinant Avipox virus in which a DNA coding for the fusion protein according to any one of claims 1 through 8 has been inserted.

12. A recombinant live vaccine for anti-fowl Mycoplasma gallisepticum infection comprising as an effective ingredient a recombinant Avipox virus in which a DNA coding for the fusion protein according to any one of claims 1 through 8 has been inserted.

13. A trivalent live vaccine for anti-fowl Mycoplasma gallisepticum infection and anti-Marek's disease infection comprising as an effective ingredient a DNA coding for the fusion protein according to any one of claims 3 and 4.

FIG.1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EcoRI
EcoT221
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SalI
KpnI
SmaI
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EcoRI
PstI
BamHI
HindIII
EcoT22I
XhoI
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HindIII
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5.65 kb
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EcoRI
SfiI
BglI
SalI
EcoRI
MluI
HindIII
MluI
BamHI
i)EcoRI
ii) Klenow
740 bp
i) XbaI
ii) Klenow
5.65 kb
BamHI
BglII
HindIII
BglI
BglI
280 bp
SspI
EcoT22I
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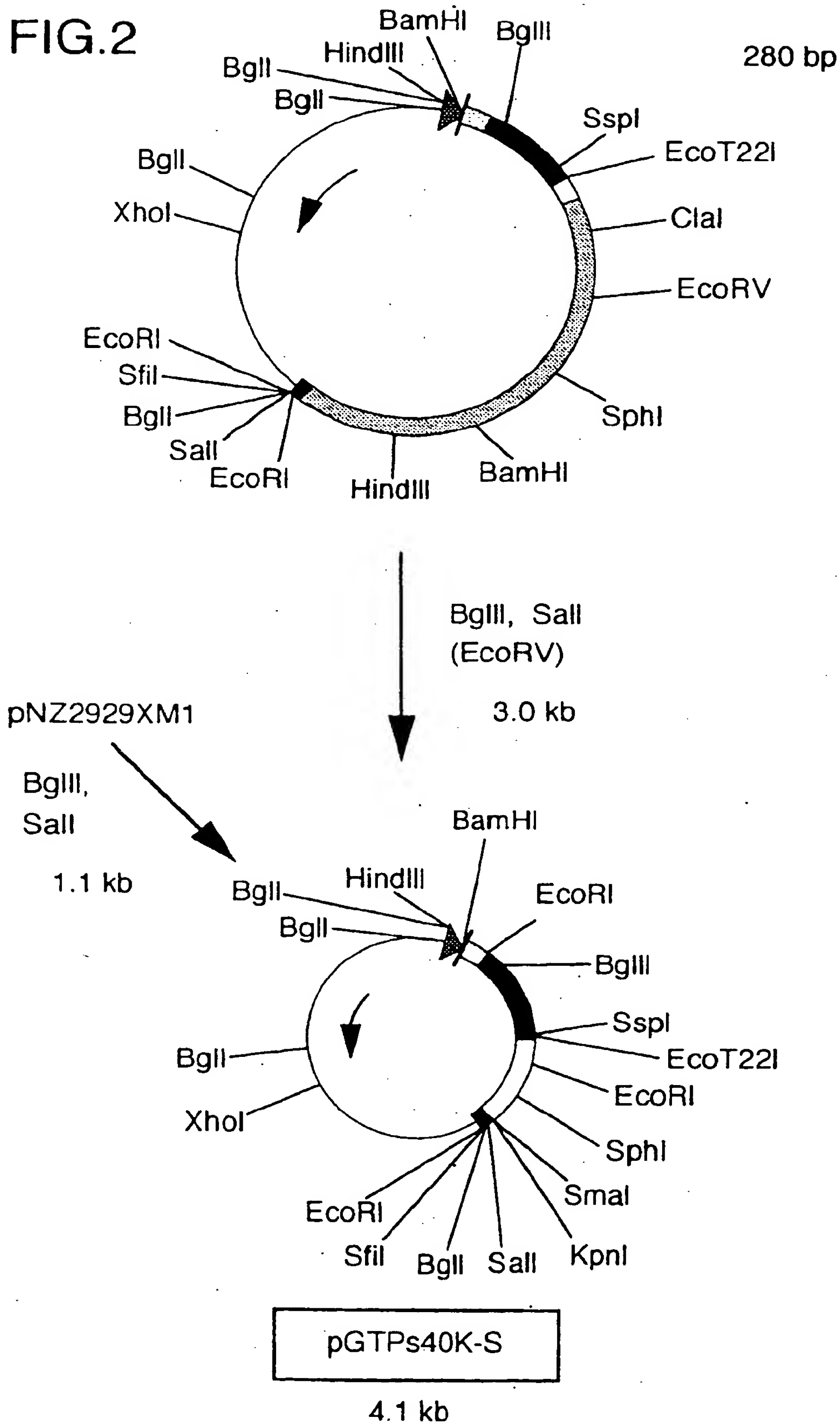
HindIII
BamHI
SphI

FIG.2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BglI
SalI
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(EcoRV)
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BglI
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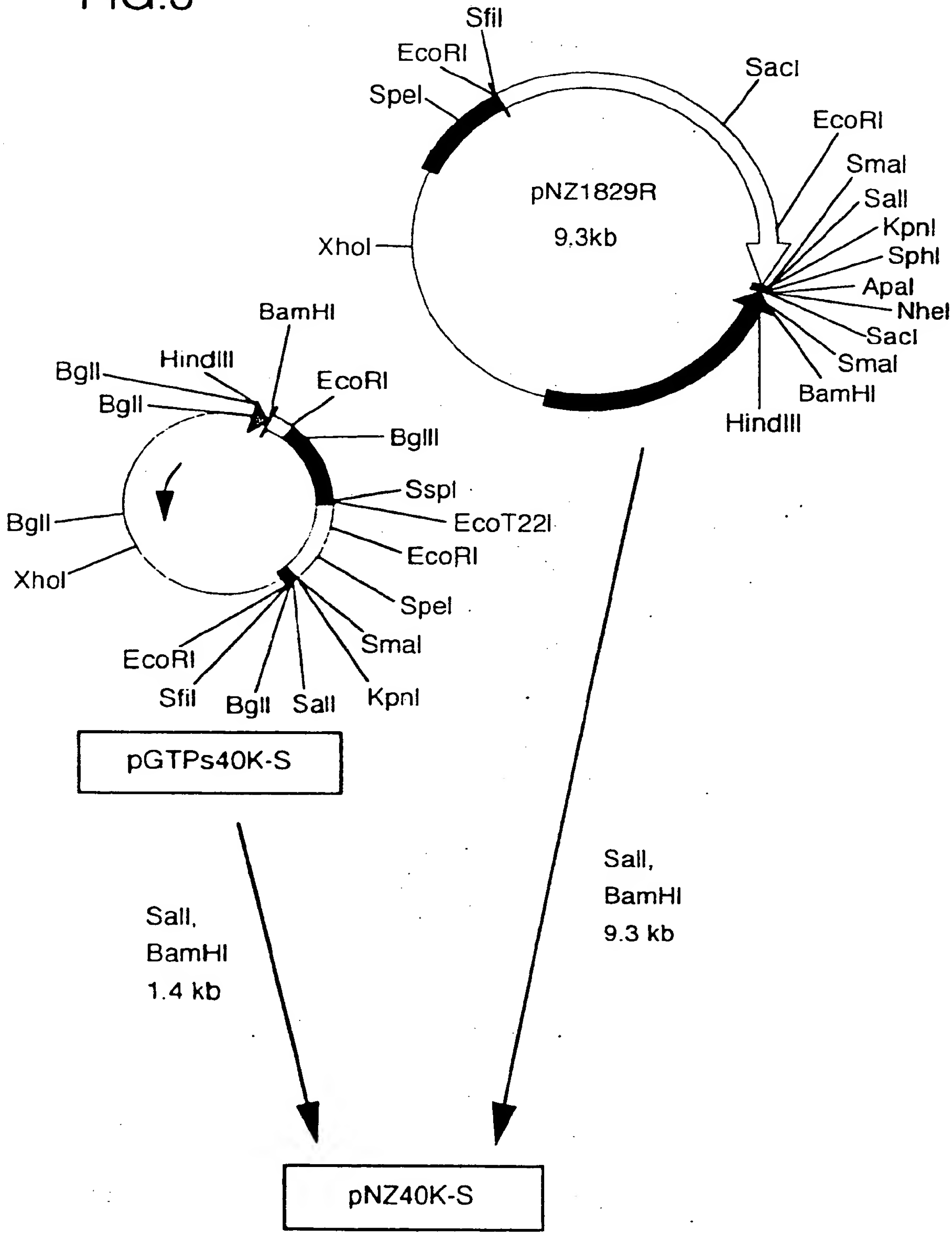
4.1 kb

FIG.3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HindIII
XhoI
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9.3kb
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BglI
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BglII
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EcoRI
SpeI
SmaI
KpnI
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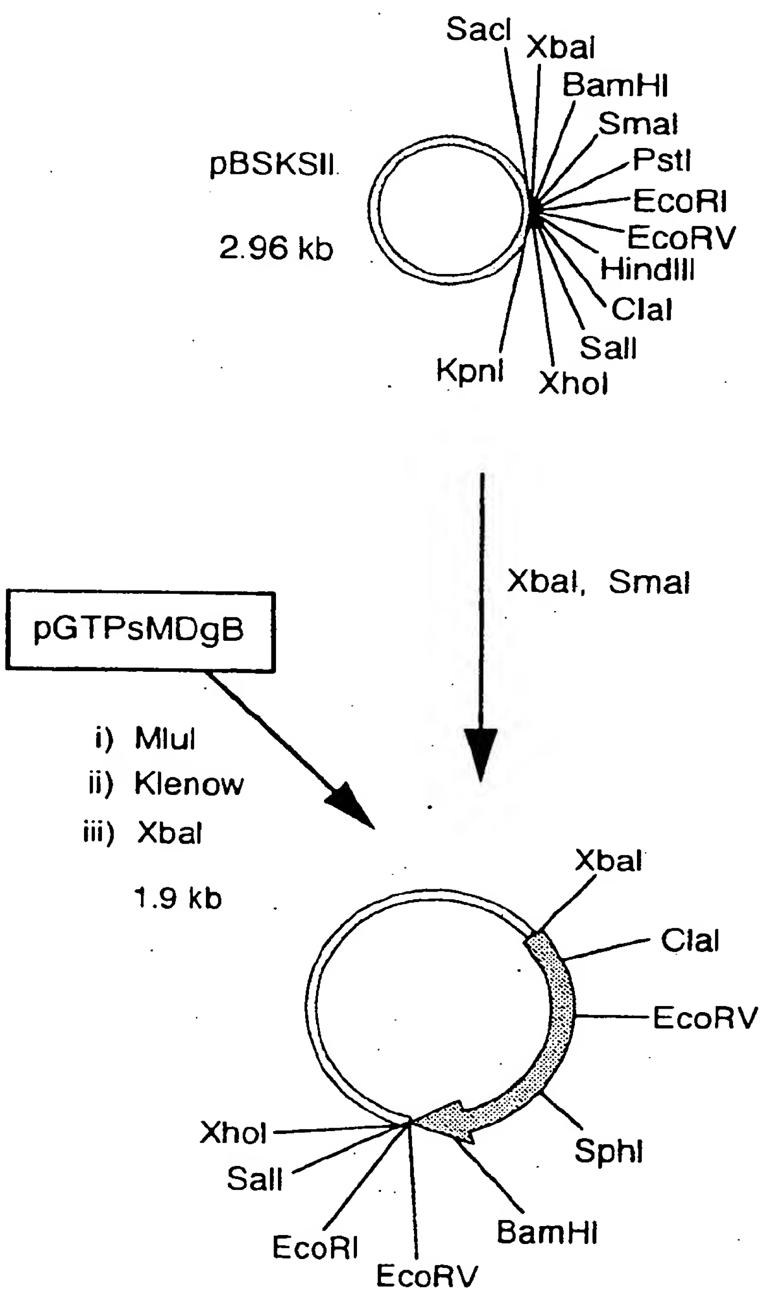
FIG.4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pGTPsMDgB
i) MluI
ii) Klenow
iii) XbaI
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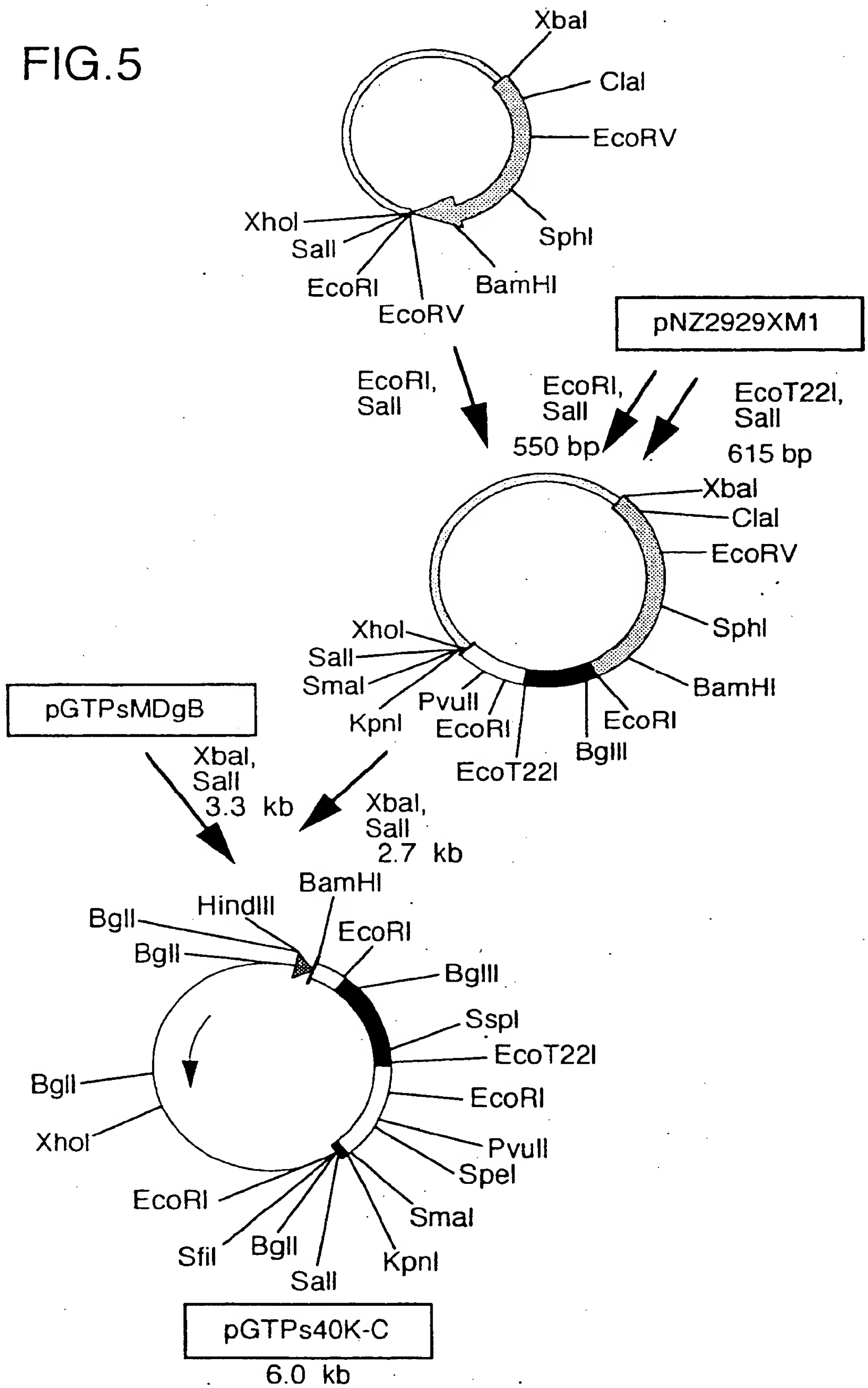
SalI
XhoI

FIG.5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SalI
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EcoT22I,
SalI
615 bp
XbaI
ClaI
EcoRV
SphI
BamHI
EcoRI
BglII
EcoT22I
EcoRI
PvuII
KpnI
SmaI
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XhoI
pGTPsMDgB
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SalI
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SalI
2.7 kb
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BglI
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BglII
SspI
EcoT22I
EcoRI
PvuII
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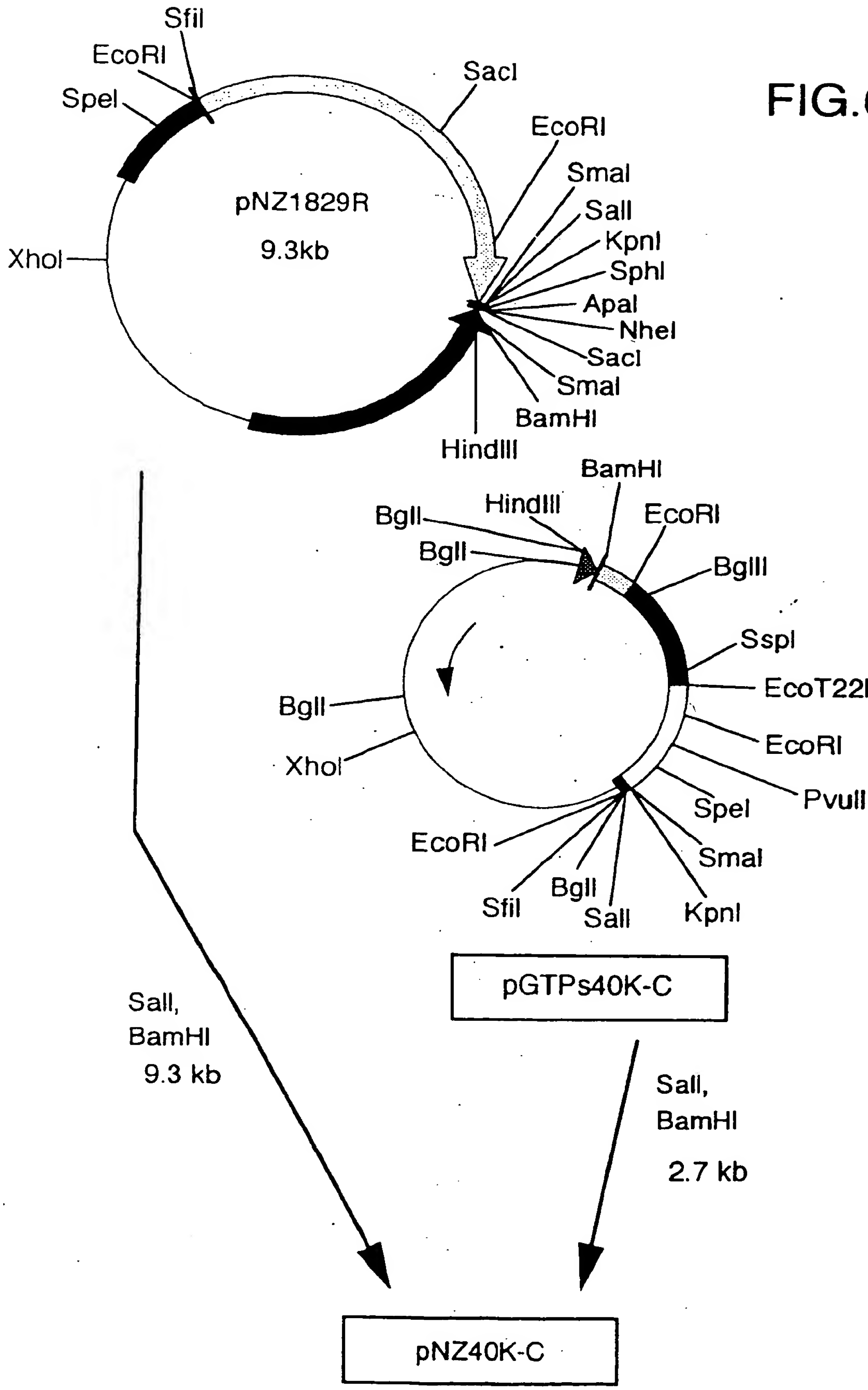
6.0 kb

FIG.6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EcoRI
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SalI
KpnI
SphI
ApaI
NheI
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XhoI
pNZ1829R
9.3kb
BamHI
HindIII
BglI
BglI
EcoRI
BglII
SspI
EcoT22I
EcoRI
PvuII
SpeI
SmaI
KpnI
SalI
BglI
SfiI
EcoRI
XhoI
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BamHI
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pNZ40K-C

FIG.7

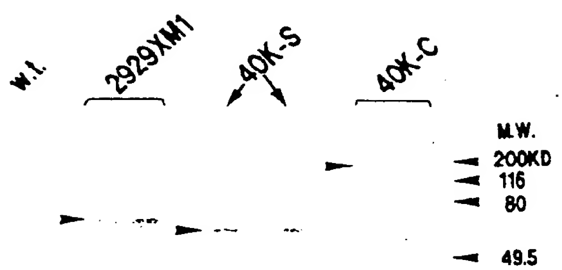
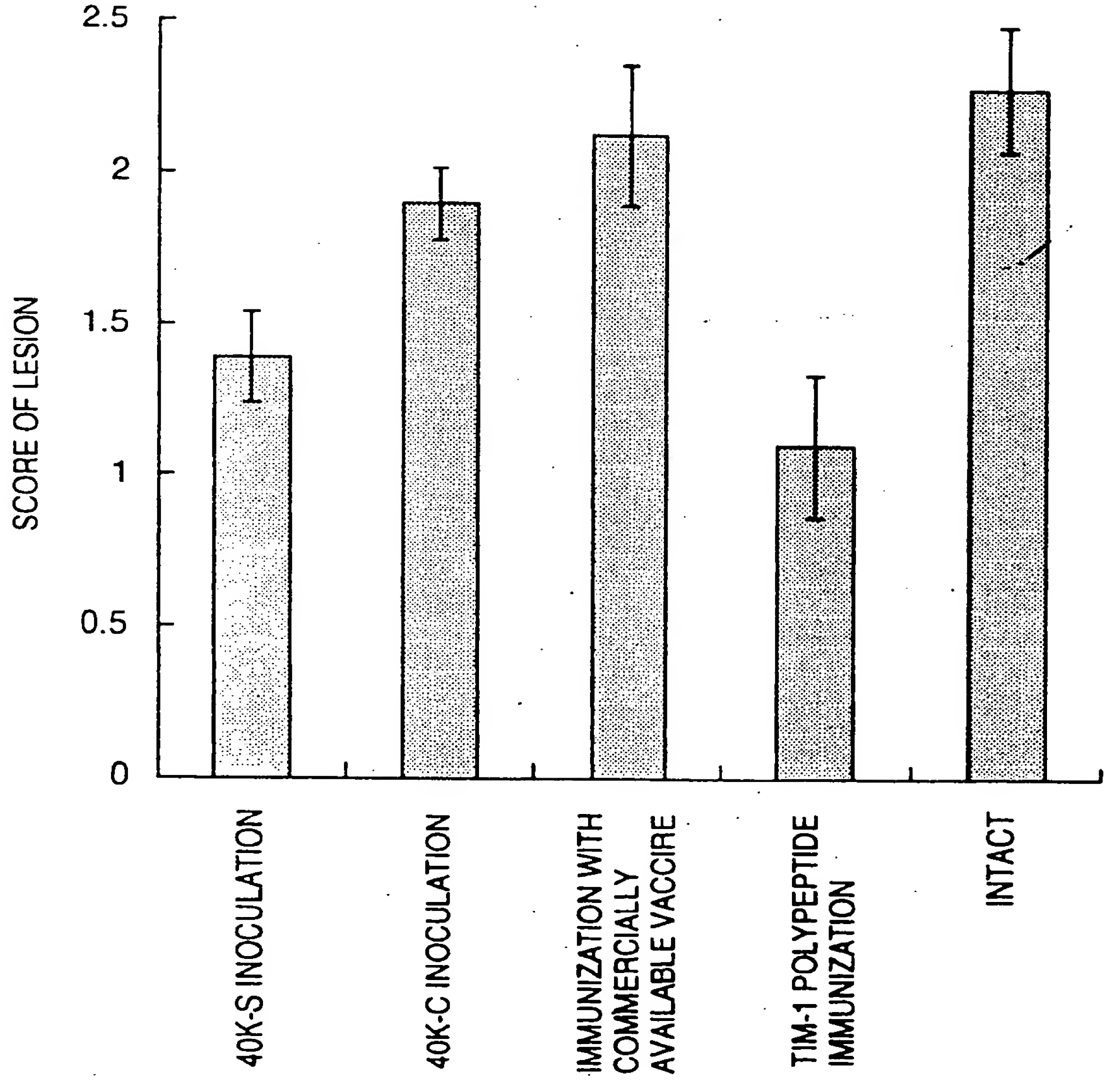


FIG.8


2.5
2
1.5
1
0.5
0
SCORE OF LESION
40K-S INOCULATION
40K-C INOCULATION
IMMUNIZATION WITH COMMERCIALLY AVAILABLE VACCIRE
TIM-1 POLYPEPTIDE IMMUNIZATION
INTACT

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/01084

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl[6] C07K14/30, 14/055, 14/065, C12N15/31, 15/38, 15/62, C12N7/01, A61K39/255 // C12P21/02, (C12P21/02, C12R1:92)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl[6] C12N15/00-15/90

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS PREVIEWS, WPI, WPI/L

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, 94/23019, A (Nippon Zeon Co., Ltd.), October 13, 1994 (13. 10. 94) & AU, 9462926, A & EP, 692532, A	1 - 13
Y	YOSHIDA, Shigeto et al. "The glycoprotein B genes of Marek's disease virus serotypes 2 and 3: identification and expression by recombinant fowlpox virus", Virology (1994) Vol. 200, No. 2, p. 484-493	1 - 13
Y	NAZERIAN, K. et al. "Protection against Marek's disease by a fowlpox virus recombinant expressing the glycoprotein B of Marek's disease virus", Journal of virology (1992), Vol. 66, No. 3, p. 1409-1413	1 - 13
Y	JP, 7-503843, A (Virogenetics Corp.), April 27, 1995 (27. 04. 95) & WO, 93/14219, A & AU, 9334353, A & EP, 623172, A	1 - 13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
June 24, 1997 (24. 06. 97)	July 1, 1997 (01. 07. 97)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/01084

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP, 5-504253, A (British Technology Group Ltd.), July 8, 1993 (08. 07. 93) & EP, 408301, A & GB, 2233655, A & WO, 91/00911, A & AU, 9059555, A	1 - 13

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